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14. ABSTRACT
Spores of Bacillus megaterium and Bacillus subtilis were harvested shortly after release from sporangia, incubated under various conditions and spore metabolism monitored by 31P-NMR of small molecules. Incubation for up to 30 d at 4-50°C in water or buffer to raise spore core pH to 7.8, or at 4° in spent sporulation medium led to no changes in small molecules including 3-phosphoglyceric acid (3PGA) or mono nucleotides, and no ATP accumulation. Similar results were obtained with spores that had gone through Stage I of germination and were incubated 8 d at 37°C. However, spores incubated in spent sporulation medium at 37 or 50° degraded much 3PGA and accumulated

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Report Title

Final Report: Measurement of Metabolic Activity in Dormant Spores of Bacillus Species

ABSTRACT

Spores of Bacillus megaterium and Bacillus subtilis were harvested shortly after release from sporangia, incubated under various conditions and spore metabolism monitored by 31P-NMR of small molecules. Incubation for up to 30 d at 4-50°C in water or buffer to raise spore core pH to 7.8, or at 4° in spent sporulation medium led to no changes in small molecules including 3-phosphoglyceric acid (3PGA) or mono nucleotides, and no ATP accumulation. Similar results were obtained with spores that had gone through Stage I of germination and were incubated 8 d at 37°C. However, spores incubated in spent sporulation medium at 37 or 50° degraded much 3PGA and accumulated mono nucleotides (but not ATP!), and these processes were accelerated when core pH was raised to 7.8. These data indicate that spores store in water or buffer at low or high temperatures exhibit little if any metabolism of endogenous compounds. However, spores stored in spent sporulation medium did exhibit some metabolism, including 3PGA degradation and mono nucleotide accumulation, the latter indicative of RNA degradation. These results indicate that "dormant" spores can carry out some metabolic reactions.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

none

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Anthony Troiano	0.50	
FTE Equivalent:	**0.50**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Peter Setlow	0.10	
Barabara Setlow	0.15	
FTE Equivalent:	**0.25**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
George Korza	0.20
FTE Equivalent:	**0.20**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

This project was undertaken to examine the level of metabolism in "dormant" spores of Bacillus species, in light of a recent report that there is much RNA degradation of ribosomal RNA when newly harvested Bacillus subtilis spores are incubated at physiological temperatures, as well as some evidence for transcription in such spores. The plan for the work was to directly examine metabolism in dormant spores incubated at 4, 37 and 50°C by using 31P-NMR to determine changes in levels of spores large depot of 3-phosphoglyceric acid (3PGA) that is used to generate ATP in the first min of spore germination, and also to examine levels of ribomononucleotides (RMN), that are predominantly AMP with smaller amounts of CMP, GMP and UMP in spores, as well as levels of ADP and ATP, as well as other ribonucleoside di-and tri-phosphates. Use of spores of strains of Bacillus megaterium and Bacillus subtilis that lacked the nutrient germinate receptors (GRs) that trigger spore germination due to nutrient germinates was crucial for this work. When wild-type spores were incubated for extended periods (days or weeks), at 37 or 50°C, the great majority of spores ultimately germinated, making assessment of metabolism in dormant spores essentially impossible. However, the GR-less spores incubated at 37 or 50°C for up to 30 d in water, buffer or spent sporulation medium exhibited less that 5% spore germination, and the germinated spores were easily removed by centrifugation on a high density solution in which dormant spores pellet, and germinated spores float.

To carry out this work, GR-less spores of Bacillus megaterium and Bacillus subtilis were harvested shortly after release from sporangia, incubated under various conditions, spore small molecules extracted, and Mn removed by Chelex chromatography, as Mn interferes with NMR analyses. Levels of phosphate-containing small molecules, and thus spore metabolism, were then measured by 31P-NMR. Incubation for up to 30 d at 4-50°C in water or buffer to raise spore core pH to 7.8, or at 4° in spent sporulation medium led to no changes in small molecules including PGA or RMN, and no detectable accumulation of ATP or other ribonucleoside triphosphates. Similar results were obtained with B. megaterium spores that retained GRs and that had gone only through Stage I of germination and had released their dipicolinic acid but could not complete germination because they could not degrade their peptidoglycan cortex layer. Compared to dormant spores, these Stage I germinated spores have an elevated core pH of 7.8 compared to 6.3 in dormant spores and a slightly elevated core water content, ~ 50% of wet wt, vs ~ 40% in dormant spores. Strikingly, incubation of these Stage I germinated spores for 8 d at 37°C in buffer degraded no 3PGA and accumulated no RMN, including ATP. However, dormant spores incubated in spent sporulation medium at 37 or 50° degraded much 3PGA and accumulated RMN (but not ATP!), and these processes were accelerated when core pH was raised to 7.8. These data indicate that spores stored in water or buffer at low or high temperatures exhibit little if any metabolism of endogenous compounds. However, spores stored in spent sporulation medium do exhibit some metabolism, including 3PGA degradation and RMN accumulation, the latter indicative of RNA degradation. These results indicate that "dormant" spores can carry out some metabolic reactions. Further work will be needed to more accurately determine the fate(s) of 3PGA that disappears from dormant spores (although it is not excreted), and the source of the RMN that accumulate in dormant spores under some incubation conditions.

The work described above is the basis of a manuscript that is in press in the Journal of Bacteriology.

Technology Transfer

None